Research on Crustaceans

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Abstract

Crustacean research at the SEAFDEC Aquaculture Department during the last three years focused mostly on the tiger shrimp *Penaeus monodon.* Studies were done along six problem areas: (1) developing spawning techniques for captive broodstock, (2) defining physico-chemical levels tolerable by larvae or postlarvae, (3) finding alternative feeds or fertilizers for extensive culture, (4) reducing the cost and evaluating the quality of formulated feeds for semi-intensive culture, (5) preventing and controlling disease, and (6) documenting the chemicals used in shrimp culture and their effects on the environment. To reduce feed costs, substitutes for expensive feed components were screened and the specific nutrient requirements of tiger shrimp during culture were determined. A few studies were made on other crustaceans. The vitellogenin levels during maturation of the white shrimp *P. indicus* were measured. The digestibility of feedstuffs was also tested in the white shrimp. Culture techniques are being developed for the mudcrab *Scylla serrata* in ponds, pens, and cages.

Introduction

Studies were conducted in 1992-1994 on three crustacean species: the tiger shrimp *Penaeus monodon,* the white shrimp *P. indicus,* and the mudcrab *Scylla serrata.* The mudcrab was identified during ADSEA '91 as the top priority crustacean for research and the white shrimp as another alternative species for grow-out in ponds. However, most of the research at AQD still focused on the tiger shrimp, the most important commercial species and the one beset with many problems.

Studies on the Tiger Shrimp *Penaeus monodon*

Breeding

Quinitio et al. (1993) correlated the ovarian development with the morphological changes of the genitalia during the first maturation of female tiger shrimp. They identified two stages, the primordial germ cell and the chromatin nucleolus stages, during the earlier phase of maturation, in addition to the four later stages seen by Tan-Fermin and Pudadera (1989). Females mate only when the thelycum is fully developed (Quinitio et al. 1993).

A most critical problem in tiger shrimp hatchery production today is the lack of spawners. Tan-Fermin (1991) compared ablated pond-reared adults, ablated wild-caught adults, and unablated wild spawners in terms of growth and reproduction. Ablation enhanced maturation but led to the retention of bigger and more advanced oocytes. Thus, ablated females released fewer eggs than unablated wild spawners. The results also suggested that ablation may induce growth simultaneous with maturation in pond-reared but not in wild-caught broodstock.

Primavera and Caballero (1992a) found that unablated females reared in low-intensity green light, or ambient light under black cover spawned the most eggs. The number of spawns increased 14-17x with ablation. In wild-caught males, unilateral ablation increased the number and spike length of sperm cells and reduced the percentage of abnormal sperm (Gomes and Primavera 1993). Ablation may thus help improve the sperm quality in pond-reared males — if these behaved like the wild males. The effect of captivity on the sperm quality of wild and pond-reared tiger shrimps is currently being studied (ET Quinitio, personal communication).

Growth and secondary sexual characteristics were studied in pond-reared broodstock. Primavera and Caballero (1994) found that joining of the petasma, presence of sperm in spermatophores in males, and sperm in the thelycum of females were first noted in shrimps 26-28 mm in carapace length. Morphometric changes and gonadal development in pond-reared broodstock are currently being studied further.

A study by Millamena et al. (1993) showed that polyunsaturated fatty acids reach very high levels during, and may have a significant role in, the maturation of ablated females. The role of carotenoids in the reproduction of pond-reared tiger shrimp was determined by comparing diets with or without astaxanthin (MPY Pangantihon, personal communication), or carotenoid-containing foods such as mussel meat, crab meat, and *Artemia* in combination with a pelleted broodstock diet (ET Quinitio, personal communication). In both studies, maturation, spawning, and hatching rates were very variable and not significantly different among treatments.

Hatchery

Physiological studies were undertaken to define the optimal rearing conditions for tiger shrimp larvae and postlarvae. Optimal salinity ranges were determined by bioassays. Of the larval stages, the nauplii show the most limited salinity tolerance. Only 50% of nauplii subjected to a salinity 4 ppt lower than the spawning salinity develop into protozoea (Parado-Estepa et al. 1993).

Ammonia and pH also affect larval growth and survival in tiger shrimp (Noorhamid et al. 1994). Almendras (1994) used ammonia excretion as an index of the tiger shrimp's physiological state when transferred to different salinities or different pH. Ammonia excretion temporarily but significantly increases after abrupt transfer from seawater to lower salinities, and decreases at higher salinities. Change in pH similarly altered ammonia excretion. High pH has a more lethal effect than low environmental pH; 40% of the postlarvae transferred to high pH started dying after 2.5 hours (Almendras 1994).

Water management techniques in the hatchery were compared in terms of the survival, growth, and development of tiger shrimp larvae (Parado-Estepa, unpublished).

The economics of shrimp hatcheries was studied by Agbayani et al. (1994). The hatchery business was less profitable in 1991 than in 1985 due to the low market price of postlarvae (Table 1). In 1991, medium-scale hatcheries (100-300 tons in water capacity) registered the highest economic returns. Small hatcheries less than 100 tons in capacity and large hatcheries more than 300 tons were more vulnerable to changes in market prices and other natural factors such as diseases, typhoons, supply of spawners, and price decreases. High survival rates improved cost efficiency in hatchery operations.

Grow-out culture

Nursery rearing of postlarvae in ponds is still practised by extensive shrimp growers. Rodriguez et al. (1993) demonstrated that nursery rearing can also be done in 'hapa' nets installed in the pond. With feeding, postlarvae can be stocked at higher densities in hapa nets without sacrificing growth and survival.

Chicken manure, rice hulls, and sugar mill wastes were tested as fertilizers in extensive shrimp ponds. In terms of growth and survival of tiger shrimps, boiler ash (derived from burned bagasse from sugar mills) was a more efficient fertilizer than chicken manure (Subosa 1992).

The aquatic weeds *Ruppia maritima* ('kusay-kusay') and *Najas graminea* ('digman') commonly found in extensive ponds were shown to provide both food and shelter for shrimps (Primavera and Gacutan 1989). The assimilation efficiency of shrimps for *Ruppia* was higher than for *Najas* and significantly higher in males than in females (Catacutan 1993). In another study, shrimps grown in ponds with *Ruppia* and with plankton had better survival and growth than those in ponds with filamentous green algae (Bombeo-Tuburan et al. 1993).

Farm-made feeds are occasionally used to supplement the natural food in the pond. Bombeo-Tuburan et al. (in press) studied the feasibility of using the golden apple snail *Pomacea canaliculata* or 'kuhol', a common pest in rice culture, as feed for tiger shrimp. 'Kuhol' given in combination with com or cassava resulted in good production and size-frequency distribution of shrimps.

Pesticides are used in extensive shrimp ponds to eliminate unwanted species that compete with the shrimps for food, oxygen, or space. Some pesticides cause mortalities or toxin-related abnormalities such as the soft-shell syndrome. The tolerance of juvenile shrimps to two commonly used pesticides in ponds, saponin in teaseed cake and rotenone in derris root, was determined in laboratory bioassays (Cruz-Lacierda 1993). Both pesticides were proven safe at the concentrations customarily used in ponds, 0.4 ppm purified rotenone and 15 ppm crude saponin. Significant mortalities and soft-shelling occurred only at concentrations greater than 1 ppm rotenone or 100 ppm saponin (1 ppm=1 mg/l).

Pond snails are often very difficult to eliminate. They are usually just handpicked since inorganic molluscicides also cause soft-shelling in shrimps. Burning rice straw piled 15 cm thick at the pond bottom effectively gets rid of snail species (Triño et al. 1993).

Table 1. Comparative technical and economic indices of small, medium, and large shrimp hatcheries in Panay in 1985 and 1991. Data from Agbayani et al. (1994). US$1=₱25; 1 peso = 100 centavos.

Indices	1985			1991		
	Small	Medium	Large	Small	Medium	Large
Number of hatcheries	10	17	11	97	40	15
Stocking rates (larvae/liter)	50	50	50	17	17	15
Number of runs/year	10	10	10	4	6	8
No. of good runs	10	10	10	3	4	6
No. of bad runs	10	10	10	1	2	2
Survival rates (%)	10	10	10	10	16	11
Production (× 1,000 postlarvae)	560	1,600	18,000	1,688	5,452	6,977
Investments (pesos)	350,000	2,754,600	4,513,852	244,387	561,111	826,216
Prices (centavos)	30	30	30	8	11	10
Revenues (pesos)	168,000	1,600,000	5,400,00	144,094	619,596	672,709
Earnings above variable cost (pesos)	285,816	1,170,152	5,393,439	34,186	220,526	107,879
Net income (pesos)	309,600	1,208,000	3,927,000	(9,732)	122,381	(25,739)
Variable cost per fry (centavos)	12	10	9	5	6	7
Total cost per fry (centavos)	13	12	10	8	8	9
Return on investment (%)	90	41	74	-4	22	-3
Return on working capital (%)	562	643	1338	30	279	35
Net present value (pesos)	781,800	1,338,000	9,000,000	105,251	460,885	292,369
Benefit/cost ratio	1.5	1.1	1.4	1.2	1.3 1.1	
Internal rate of return (%)	123	29	55	52	100	63

Primavera (1993) compared the different shrimp farming systems in terms of resource use, effluents, economics, and effects on the environment. Semi-intensive shrimp culture is ecologically and economically more viable than extensive or intensive culture and is recommended for sustainability of the industry in the Philippines.

Searanching or restocking of juvenile shrimps back in the wild is being planned by SEAFDEC AQD. One initial study has been done on the tagging of shrimps since the success of searanching can only be evaluated by monitoring the performance of released animals. Primavera and Caballero (1992b) showed that streamer tags do not cause immediate mortality in 2 or 7 month old tiger shrimps in tanks, but significant mortality occurs after 6-8 weeks of tagging.

Feed development

Feeds account for 50-60% of total expenses in semi-intensive shrimp culture. Thus, many AQD studies were directed towards lowering the cost of feeds for tiger shrimp. Three major approaches were tried: (1) determination of the nutrient requirement and optimal levels for tiger shrimp, (2) screening of substitutes for expensive feed components such as fish meal and imported binders, and (3) finding alternative sources of protein or lipids.

The requirement of tiger shrimp for threonine is 1.45% of the diet or 3.5% of dietary protein (Millamena et al., in press). Postlarvae also showed best growth and survival on diets with arginine at 4.6% of dietary protein and with valine at 5.8% of dietary protein (OM Millamena, personal communication).

Catacutan and Lavilla-Pitogo (1994) found that 50-100 ppm ascorbic acid or 100-200 ppm phosphated ascorbic acid, a more stable derivative, is needed for good growth and survival of juveniles. These levels improve the structure of the hepatopancreas of shrimps infected with monodon baculovirus. Nevertheless, pond studies indicate that there is no need to include vitamins in shrimp diets for semi-intensive culture as sufficient vitamins may be supplied by the natural food in ponds (Triño et al. 1992).

Fish meal is not just expensive; it also competes with human consumption of fish and is ecologically inefficient in that it adds one step to the food chain of cultured animals. Thus, there is a great need to find less costly or more abundant sources of protein. Lactic yeast and fish protein concentrate were tested and found to be good alternative sources of protein (Millamena, in press). Leaf meals such as those from kamote *Ipomoea batatas,* kangkong *I. aquatica,* malunggay *Moringa oleifera,* and papaya *Carica papaya* were tested as partial replacement for fish meal (VD Penaflorida, personal communication).

Various legumes were also tested as protein sources and methods were developed to remove toxic components and increase digestibility. *Leucaena leucocephala* or 'ipil ipil' was shown to be a promising substitute for fish meal, but its use is limited by the presence of mimosine, an anti-nutritional factor (Pascual and Catacutan 1990). Peñaflorida et al. (1992) found that soaking for 24 hours was a practical and effective method to remove mimosine from mature 'ipil-ipil' leaves. Eusebio (1991) found that dehulling increased the digestibility of rice bean, but not of cow pea used in diets for tiger shrimps. Dehulling removes the tannins that occur mostly in the seed coats.

Wheat flour is commonly used as binder in feeds. It is relatively expensive because wheat is not locally grown. Since carbohydrates from red seaweeds may also have similar binding quality as flour, meals from *Kappaphycus* and *Gracilaria* were tested as binders for shrimp diets. It was found that *Gracilaria* can be used at 10% of the diet weight to replace 67% of the wheat flour (VD Peñaflorida and NV Golez, personal communication).

Alternative lipid sources were also tested. Cod liver oil, soybean oil, and corn oil proved to be good sources of lipid, but beef tallow, pork lard, and coconut oil resulted in poor growth and survival of tiger shrimp (Catacutan 1991).

Juvenile tiger shrimps tolerate fat oxidation or malonaldehyde levels equivalent to 828 mg TBA/kg diet (PF Subosa, personal communication; TBA is thiobarbituric acid, an indicator). The effects of antioxidants and storage conditions on feed quality were studied. It was found that antioxidants need not be added to freshly prepared feeds that are used within 1-2 months and stored at 28-30°C (Bautista et al. 1992). Under such conditions, shrimps fed diets without antioxidants survive and grow as well as those fed diets with antioxidants, and the feeds without antioxidants show no significant signs of deterioration. However, inclusion of the antioxidant butylated hydroxytoluene in feeds stored up to 4 months improves the growth rate of juvenile shrimps (PF Subosa, personal communication).

Some shrimp diseases may be due to fungal toxins formed during deterioration of feeds in storage. A survey revealed that commercial shrimp 'finisher' feeds in the Philippines had aflatoxin B_1 levels ranging from 'not detected' to 120 µg/kg, and most feeds contained 10-40 µg/kg (Bautista et al. 1994). Studies then determined the tolerance of tiger shrimp to fungal toxins in deteriorating feeds. Pre-adult tiger shrimp (17 grams) tolerate aflatoxin B_1 up to 52.3 (µg/kg without any change in growth, but histopathological changes already appear at 26.5 µg/kg aflatoxin B_1 (Bautista et al. 1994). Similarly, 5 gram juveniles tolerate up to 50 ppb aflatoxin B_1 (Bautista and Subosa, in press).

Diseases

Luminescent vibriosis is a common fatal disease caused by the bacterium *Vibrio harveyi* that invades shrimp hatcheries. Lavilla-Pitogo et al. (1992) studied the sources of *V. harveyi* in tiger shrimp hatcheries. To determine whether the disease can be controlled by environmental manipulation, Lavilla-Pitogo (personal communication) determined the tolerance of *V. harveyi* to various temperatures, pH, and salinities. The bacteria were found to be tolerant of a wider range of conditions than tiger shrimp larvae or postlarvae. Elimination of the natural microflora enhanced the survival of *V. harveyi* in hatchery tanks and the presence of the diatoms *Chaetoceros* and *Skeletonema* effectively controlled it (CR Lavilla-Pitogo, personal communication).

Studies were also conducted on the role of *Vibrio* spp. in the etiology of red disease syndrome (E Tendencia, personal communication) and the incidence and etiology of the swollen hind-gut syndrome (CR Lavilla-Pitogo, personal communication) in tiger shrimps.

Numerous drugs and chemicals are used to control diseases in aquaculture in the Philippines (Baticados and Paclibare 1992). Another survey found that about 40 chemical and biological products are used in intensive shrimp farms (Primavera et al. 1993). Table 2 shows these products. Both studies strongly recommend that medically important antibiotics not be used in aquaculture because antibiotic-resistant strains of human pathogens may develop. Government is urged to regulate both the use of bioactive compounds and the disposal of pond wastes.

Table 2. Chemical and biological products used in hatcheries and grow-out ponds of the shrimp *Penaeus monodon* in the Philippines. Modified from Primavera (1993).

Product	Use	Remarks
Therapeutants		
Chloramphenicol	Antibacterial	Commonly used in hatcheries; added to feeds by some farmers
Doxycyline	Antibacterial	
Erythromycin	Antibacterial	Used as hatchery bath
Formalin	Fungicide, parasiticide	For necrotic shell and gill diseases; also for spawner disinfection
Malachite green	Parasiticide, antifungal	Used as bath or added to pond system
		Against shell and gill diseases; for disinfection of eggs
	diseases;	
Nitrofurans (furazolidone, nifurpirinol)	Antibacterial, fungicide	Disinfection of spawners; may cause deformities in larvae
Oxolinic acid	Antibacterial	Added to grow-out feeds
Oxytetracycline	Antibacterial	Added to feeds or to ponds directly
Rifampicin	Antibacterial	Used in hatcheries; prescribed for
	human tuberculosis	
Sulfa drugs	Antibacterial	Added to feeds
Trifluralin	Antifungal (Treflan)	Agricultural herbicide used in hatcheries
Disinfectants		
Calcium sulfide and calcium hypochlorite commercial bleach		Widely used in hatcheries
EDTA	Chelates heavy metals	Hatchery use
Iodine compounds	For disinfection	Used against shell-related diseases
Laundry detergent	For disinfecting eggs	
Alkyl benzyl dimethyl ammonium chloride	Antibacterial, antifungal	Used for soil and water treatment
Benzalkonium chloride	Antibacterial	Also used to induce molting
Dodecyl dimethyl ammonium bromide	Antibacterial, antifungal	

Table 2 Continued.

Product	Use	Remarks
Soil and water treatment		
Bacteria-enzyme preparations	Decomposition of organic matter	Used in the wastewater treatment industry
Lime $Ca(OH)_2$ $CaCO_3$ CaO	Traditional pond inputs Increases pH, disinfects	Also used to induce molting
Potassium permanganate	Oxidizer and detoxifier	
Zeolite	Absorbs toxic gases	For water quality maintenance
Pesticides		
Ammonium sulfate	Piscicide	Used with burnt lime
Chelated copper	Algicide, antibacterial	Also used to induce molting
Teaseed powder, crumble, flakes	Piscicide	Widely used in ponds; also used to induce molting
Plankton growth		
Chemical fertilizers urea (45-0-0) and monoammonium phosphate (16-20-0)	Traditional pond inputs	
Organic fertilizers dried chicken manure, others	Traditional pond inputs	
Nutrient mixes		May be mixed with fertilizers
Feed additives (two or more may be combined in one product)		
Calcium, other minerals		
Vitamin C, others		
Enzymes		
Hormones		
Fatty acids		
Protein extracts		
Chicken eggs (binder)		

Tiger shrimps may also be exposed to heavy metals in addition to the chemicals listed in Table 2. Juveniles were found able to detoxify and remove copper and lead by granule formation and excretion; thus, tiger shrimps, like other decapods, are not suitable for biomonitoring of heavy metal pollution (Vogt and Quinitio 1994).

Ecology

The role of mangroves as shrimp nurseries for juvenile shrimps is currently being studied (J H Primavera, personal communication). A riverine mangrove and an island mangrove were selected as study sites. The shrimp populations at the two sites include *Penaeus merguiensis, P. monodon, P. latisulcatus, P. semisulcatus,* and at least four *Metapenaeus* species. These shrimps usually lie hidden during the day and emerge to feed at night. Carbon isotope ratios showed that mangrove detritus is not the primary food source of juvenile shrimps at the two sites (JH Primavera, personal communication).

Studies on the White Shrimp *Penaeus indicus*

The white shrimp is commonly harvested as an incidental species from tiger shrimp ponds. Larval rearing is like those of other penaeids, but the reproductive physiology has not been studied. Techniques for the grow-out of white shrimp have not been established, thus its monoculture is not widely practised. The white shrimp was identified as a priority species for research during ADSEA '91.

Quinitio and Millamena (1992) correlated the levels of vitellogenin, a female-specific protein in the hemolymph, with gonad maturation stage. The vitellogenin level is low during the immature stage, increases gradually, and reaches a peak during the mature stage.

The number of eggs spawned by white shrimps from the wild was found to increase with spawner size (CT Villegas, personal communication). Selective breeding studies on shrimps have been started.

Pond grow-out of white shrimp is a problem because of its slow growth and low survival upon reaching the size range 11-15 grams. A feed is being developed for white shrimp, starting with a study of the digestibility of 21 locally available plant protein sources. Mungbean was well digested by white shrimp enzymes *in vitro,* but resulted in low survival and growth when included in formulated diets (PS Eusebio, personal communication). Papaya leaf meal was as digestible as shrimp head meal and Peruvian fish meal.

Studies on the Mudcrab *Scylla serrata*

The mudcrab has very high market potential and many farmers have ventured into culturing or fattening them. Samonte and Agbayani (1991) demonstrated that culture of mudcrab has a short payback period of 1-2 years. Juvenile crabs stocked at 5,000/ha gave a 133% return on equity, 66% return on investment, and very promising in terms of other discounted indicators. Stocking densities of 15,000/ha and 20,000/ha were not economically feasible (Samonte and Agbayani 1991).

Techniques for grow-out culture of mudcrabs in ponds, pens, and floating cages were tested (AT Triño, personal communication). The abundance and size distribution of juvenile mudcrabs in mangrove and non-mangrove areas in northern Panay were studied (NB Solis, personal communication).

The SEAFDEC Aquaculture Department will undertake collaborative studies with the Australian Centre for International Agricultural Research and the Bribie Island Research Institute on seed production, the main bottleneck in mudcrab culture. Different feeding and water management schemes will be tested for broodstock and larvae.

Recommendations

Research on broodstock development of tiger shrimp, including genetic selection, must continue until an adequate supply of good quality spawners is assured. The cost of tiger shrimp production must be lowered, either through cheaper feeds or more efficient culture techniques. Disease prevention and control remain crucial to success. But even more important is to address the interaction between aquaculture and the environment. Environment-friendly methods of producing tiger shrimp are imperative. The negative effects of shrimp culture on the environment must be prevented, and ameliorated, otherwise, diseases will continually threaten the future of the shrimp industry.

Seed supply seems to be the main problem in the expansion of mudcrab aquaculture. Thus, research in mudcrab breeding and larval rearing must be pursued. Nutritional studies must also be conducted to develop feeds for mudcrabs in pond culture.

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